Pharmacognosy Research [Phcog Res.]
Vol 1, Issue 2, Mar-Apr, 2009 Page 91-97
[Rapid Publication]
A Supplement to Pharmacognosy Magazine – [Phcog Mag.]
Rapid publication process initiated: 18th January, 2009
Accepted: 14th February, 2009

PHCOG RES.: Research Article

New secoiridoids from *Ligustrum ovalifolium* and their hypotensive activity

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ABSTRACT
New Secoiridoid glucosides **1-5**; ligustaloside A methyl hemiacetal **1**, ligustaloside B methyl hemiacetal **2**, ligustaloside A dimethyl acetal **3** ligustaloside A butyl-methyl acetal **4**, and ligustaloside B butyl hemiacetal **5** have been isolated from the leaves of *Ligustrum ovalifolium L.* along with five known secoiridoids; ligustaloside B dimethyl acetal **6**, 10-hydroxy oleuropein **7**, 10-hydroxy ligstroside **8**, oleuropein **9** and ligstroside **10**, one known iridoid; loganin **11** and two known phenethyl alcohol glucosides; 3,4-di hydroxyphenylethyl-*β*-D-glucoside **12** and *p*-hydroxyphenylethyl-*β*-D-glucoside **13**. The structures have been elucidated by spectroscopic means including 1D NMR (^{1}H, ^{13}C, and DEPT), 2D NMR (COSY, HMQC and HMBC experiments), UV, IR and FAB-MS (positive mode). Both *n*-BuOH fraction and the isolated new compounds **1-5** were evaluated for their hypotensive activity on experimental animal. The *n*-BuOH fraction showed good activity; however, the activity of the isolated new compounds **1-5** did not particularly strong.
KEY WORDS: *Ligustrum ovalifolium*; Oleaceae; Secoiridoid glucosides; Iridoid glucosides and Phenylethanoids

INTRODUCTION

Ligustrum ovalifolium L (Oleaceae) is an evergreen shrub that is cultivated as an ornamental plant in Egypt. A literature survey indicates that this plant elaborates a number of secoiridoid glucosides i.e. oleuropein, 10-hydroxy ligstroside, ligustalosides A and B and ligustaloside B dimethyl acetal, iridoid glucosides i.e. auroside and lamaide, phenylpropanoids, phenylethanoids, lignans, flavonoids, sterols and triterpenes (1-4). The present paper describes the structure elucidation of the new secoiridoid glucosides **1-5** isolated together with five known secoiridoid glucosides **6-10**, one known iridoid glucoside **11** and two known phenethyl alcohol glucosides **12** and **13**, from the leaves of *Ligustrum ovalifolium*. Compounds **6, 8** and **9** were already found in *L. ovalifolium* (1, 2). Identification of all known compounds **6-13** were performed by comparison of spectroscopic data (UV, IR, FAB-MS, ^{1}H and ^{13}C NMR) with reported data in the literature (5-12). The hypotensive effect on anaesthetized cats was determined for the *n*-BuOH fraction and the isolated new compounds **1-5**, which showed different degrees of activities.

MATERIAL AND METHODS

General: UV spectra were determined with a Hitachi 340 spectrophotometer; IR spectra were carried out on a Nicolet 205 FT IR spectrometer connected to a Hewlett-Packard Color Pro. Plotter. The^{1}H- and ^{13}C-NMR measurements were obtained with a Bruker NM spectrometer operating at 600 MHz (for ^{1}H) and 125 MHz (for ^{13}C) in CD_3OD solution, and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. ^{13}C multiplicities were determined by the DEPT pulse sequence (135°). COSY, HMQC and HMBC NMR experiments were carried out using a Bruker AMX-600 high field spectrometer equipped with an IBM Aspect-2000 processor and with software VNMR version 4.1 or NUTS program for NMR. FABMS was taken on a VGZAB-HF reversed geometry (BE configuration, where B is a magnetic sector and E is an electrostatic analyzer) mass spectrometer. Polyamide (ICN Biomedicals), and Si gel (Si gel 60,

Merck), were used for open column chromatography. Flash column liquid chromatography was performed using J.T. Baker glassware with 40 µm Si gel (Baker) and Sepralyte C_{18} (40 µm) as the stationary phase at flow rate of 5 ml min^{-1} under N_2 pressure. TLC was carried out on precoated silica gel 60 F_{254} (Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating in dry oven at 100 for 5 min.

Plant Material:

The plant material (leaves) was collected from Zoological Garden, Giza, Egypt in March, 2006. The plant was kindly identified by Engineer Badia Hassan Aly Dewan, Consultant of Egyptian Flora, Agricultural Museum, Dokki, Giza, Egypt. A voucher specimen has been deposited in the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Material used for the determination of hypotensive effect:

Adrenaline, pentobarbitone, , propranolol, pheniramine, cimetidine, atropine sulphate and regitine. Were purchased from Novartis TM and El Nasr Pharmaceical Chemical Co. Cairo Egypt).

Extraction and Isolation:

The air dried plant material (700 g) was ground into powder and extracted exhaustively with MeOH (3 x 4 L). The combined methanolic extracts were concentrated *in vacuo* at 40^0 to dryness (152 g). The residue was dissolved in H_2O (500 ml) and filtered through Celite. The filtrate and washings were combined and defatted with petrol. The concentrated defatted crude extract (65 g) was partitioned with H_2O and *n*-BuOH. The *n*-BuOH extract (35 g) was coarsely fractionated over a Polyamide column (120 x 5 cm), and was eluted initially with H_2O, followed by addition of MeOH in 25% increments, to yield five main frs (A: H_2O; B: 25% MeOH; C: 50% MeOH; D: 75% MeOH; E: MeOH). Fr. A (9.2 g) was rechromatographed on Silica gel with $CHCl_3$-MeOH-H_2O (80:20:1-15:10:1) to give 6 frs (A1-A6). Frs A1, A2 and A3 were separately subjected to a series of flash CC at 5 ml min^{-1} under N_2 pressure [Stationary phase: silica gel for flash CC, 40 µm, eluting successively with $CHCl_3$ and a gradient of $CHCl_3$-MeOH (9:1-3:7), $CHCl_3$-MeOH-H_2O (80:10:1-70:30:3)]: flash CC at 2 ml min^{-1} under N_2 pressure [Stationary phase: Sepralyte C-18, 40 µm, using an H_2O-MeOH gradient solvent system (5→45%)]: and final purification by gel filtration CC over Sephadex LH 20 eluted with MeOH to give compounds **1** (20 mg), **2** (22 mg), **3** (26 mg), **4** (22 mg), **5** (24 mg), **6** (16 mg), **7** (25 mg), **8** (30 mg), **9** (70 mg) and **10** (52 mg). Fr. B (6.5 g) was subjected to the same series of chromatographic procedures to give compounds **11** (22 mg), **12** (49 mg) and **13** (62 mg).

***Preparation of the n-BuOH fraction and isolated new compounds 1-5*:**

The *n*-BuOH fraction (1.5 gm) was dissolved in 50 ml distilled water and 15 mg of each compound was separately suspended in 10 ml distilled H2O.

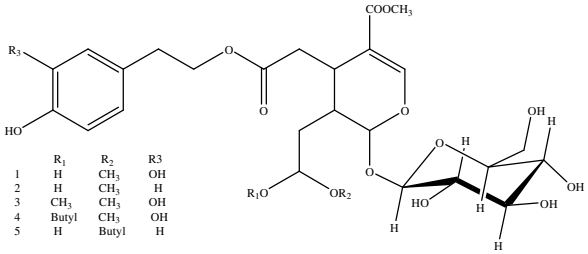
***Method for the determination of hypotensive effect*(13):**

Cats weighing 3- 4.5 Kg were used; pentobarbitone in a dose of 1 mg/kg has been used for induction and maintenance of anaesthesia. The animal was fixed to a kymograph table. Exposure of the trachea and tracheotomy were done and a Y-shaped tracheal tube was inserted and fixed in the trachea. The two limbs of the tube were connected to a pump for artificial respiration. The right femoral vein was exposed and cannulated for injection of drugs; the right carotid artery was exposed and cannulated. A mercury manometer was connected to record the blood pressure on a smoked paper, the connecting rubber tube was filled with 2% sodium citrate solution through a side tube leading to a bottle containing this solution to prevent clotting of the blood passing to the manometer. Normal tracing of blood pressure was recorded, the extract or pure compounds were injected in different doses and blood pressure changes were recorded. The femoral vein was also exposed, cannulated and connected to a burette containing saline solution just to keep the vein open for administration of drugs.

RESULTS AND DISCUSSION

***Structure elucidation*:** Compound **1**, was obtained as a white amorphous powder. Its FAB mass spectrum showed a $[M+Na]^+$ peak at *m/z* 611, corresponding to the molecular formula C_{26} H_{36} O_{15}. It showed UV maxima at 235 and 284 nm and IR bands at 3375, 1715, 1625 and 1520 cm^{-1}. These spectral data suggested the presence of an enol-ether system conjugated with a carbonyl group that was typical of secoiridoid nucleus (14), in addition to the presence of aromatic moiety. The ^{1}H NMR spectrum of **1** [Table 1], exhibited two hemiacetalic proton signals at δ 5.43 (d, *J* = 7.8 Hz, H-1) and 4.69 (d, *J* = 7.8 Hz, H-1`), proton signal of the carboxyenolic chromophore at δ 7.47 (brs, H-3) and a carbomethoxy group signal at δ 3.64 (s, OCH_3-11) due to the secoiridoid glucoside moiety and an ABX-spin system at δ 6.67 (d, *J* = 1.8 Hz, H-4``), 6.69 (d, *J* = 8.4 Hz, H-7``) and 6.55 (dd, *J* = 8.4/1.8 Hz, H-8``) together with a triplet at δ 2.77 (*J* = 7.2 Hz, $H_2$-2``), a

doublet of doublet and a doublet of triplet at δ 4.14 and 4.20 (*J* of each = 10.2/7.2 Hz) for H-1_a`` and H-$1_b$``, respectively, due to the 3,4-dihydroxyphenylethyl moiety. The ^{1}H NMR spectrum of **1** was similar to that of ligustaloside A (5) except for the absence of signal due to an aldehydic function and the appearance of a new hemiacetalic proton signal at δ 4.75 (dd, *J* = 10.2/5.4 Hz) and one methxyl signal at δ 3.34. The hemiacetalic proton was attributed to H-10 which correlated with H-8_a (δ1.60, m) and H-8_b (δ1.80, m) as shown from the cross peaks in the COSY spectrum. Furthermore H-8_a and H-8_b was further correlated with H-9 (δ2.10, m) which in turn correlated with H-1 (δ 5.43) and H-5 (δ 3.22, m). HMBC spectrum of **1** indicates the position of the additional methoxyl group to be at C-10 from the long-range correlation observed between the methoxyl protons at δ 3.34 and C-10 at δ 104.20 and between H-10 and the carbon of the methoxyl at δ 54.00. These observations suggested that **1** differed from ligustaloside A only in the moiety consisting of C-8-C-10 and possessed a C-10 hemiacetalic group. Furthermore, the ^{13}C NMR spectrum of **1** [Table 2], showed signals of C-8 and C-10 in the upper field in comparison with the corresponding signals of ligustaloside A (5).This established the structure of **1** to be a methyl hemiacetal derivative of ligustaloside A.



	R_1	R_2	R3
1	H	CH_3	OH
2	H	CH_3	H
3	CH_3	CH_3	OH
4	Butyl	CH_3	OH
5	H	Butyl	H

Compound **2**, was obtained as a white amorphous powder. Its UV and IR spectra exhibited absorption bands similar to **1**. The FAB mass spectrum showed a pseudomolecular ion peak at *m/z* 595 [M+Na] $^+$, compatible with the molecular formula C_{26} H_{36} O_{14}. Its ^{1}H and ^{13}C NMR spectra were identical to those of **1**, except for the phenylethyl moiety protons and carbons. The ^{1}H NMR spectrum of **2** showed one triplet at δ 2.83 (2H, *J* = 7.2 Hz, H_2-2``), one doublet of doublet at δ 4.16 (1H, *J* = 10.2/ 7.2 Hz, H-$1_a$``), one doublet of triplet at δ 4.20 (1H, *J* = 10.2/ 7.2 Hz, H-1_b``), and the AA`BB` signal pattern at δ 6.72 (2H, d, *J* = 8.4 Hz, 5``,7``- H) and δ 7.06 (2H, d, *J* = 8.4 Hz, 4``,8``- H), indicates the presence of a *p*-hydroxyphenylethyl moiety (5). These data implied that the structural relationship between **1**and **2** was the same as that between ligustaloside A and ligustaloside B (5). Therefore **2** was assumed to possess a *p*-hydroxyphenylethyl moiety instead of 3,4-dihydroxyphenylethyl moiety of **1**. This was established from FAB-MS which indicated that **2** was lower than **1** by 16 mass units. The ^{13}C NMR spectral data [Table 2] was assigned on the bases of comparison with those of **1** which revealed close correspondence in every aspect that the signals of 3, 4-dihydroxyphenylethyl moiety were missing for **2**. Thus the structure of **2** was established as ligustaloside B methyl hemiacetal.

Compound **3**, obtained as a white powder. The FAB mass spectrum showed a pseudomolecular ion at *m/z* 625 [M+Na] $^+$ indicating its [M] $^+$ to be 602 which corresponds to the molecular formula C_{27} H_{38} O_{15}. This established that **3** have 14 mass units more than that of **1**, indicating **3** have additional methyl group versus **1**. The UV and IR spectra indicated the presence of hydroxyl groups and a conjugated carbonyl group, which are characteristics of many iridoid and secoiridoid glucosides (13). Compound **3** was also presumed to be a ligustaloside A derivative, from the similarity of its UV, IR and NMR spectral data to those of **1**. Its ^{1}H and ^{13}C NMR [Tables 1and 2], exhibited signals due to the secoiridoid glucoside moiety and signals due to 3,4-dihydroxyphenylethyl moiety were in good agreement with the corresponging signals of **1**. The concerted use of 1D (^{1}H and ^{13}C NMR) and 2D (COSY, HMQC and HMBC) data in **3** showed the existence of two methoxyl groups [(δ_{3H} 3.310 and 3.316 each singlet) (δ_C 53.52 and 54.06)] linked to position C-10 of the secoiridoid aglycon moiety, as well as one methoxyl group [δ_{3H} 3.64, s, δ_C 51.82] which was found to be linked to position C-11 of the same aglycon. To assign the point of attachment of the two former methoxyl groups to C-10 position, the following correlations were observed in the HMBC spectrum; the methane proton (δ 4.61) of position C-10 gave cross-peaks with the two methoxyl carbons (δ 53.52 and 54.06), while the two methoxyl protons (δ 3.310 and 3.316) showed correlation with the same acetalcarbinol carbon (δ 104.50, C-10). From the above mentioned data, compound **3** was established to contain an acetalic carbon at C-10 position instead of a hemiacetalic carbon as in compound **1**. Therefore compound **3** was characterized as ligustaloside A di-methyl acetal.

Compound **4**, was obtained as a white amorphous powder with the molecular formula C_{30} H_{44} O_{15} as indicated from the FAB-mass spectrum ion at *m/z* 667 $[M+Na]^+$, suggesting that in **4** a C_4H_8 moiety was present in addition to the skeleton present in **1**. It

Table 1. ^1H NMR spectral data of compounds 1-5 (600 MHz, CD_3OD).

Position	1	2	3	4	5
Secoiridoid moiety					
1	5.43, d, 7.8	5.43, d, 7.8	5.42, d, 7.8	5.42, d, 7.8	5.42, d, 7.8
3	7.47, brs	7.47, brs	7.47, brs	7.47, brs	7.47, brs
5	3.22, m	3.22, m	3.22, m	3.23, m	3.23, m
6_a	2.44, dd, 15.0/9.6	2.44, dd, 15.0/9.6	2.48, dd, 15.0/9.6	2.47, dd, 15.0/9.6	2.44, dd, 15.0/9.6
6_b	2.52, dd, 15.0/4.5	2.51, dd, 15.0/4.5	2.52, dd, 15.0/4.5	2.52, dd, 15.0/4.5	2.51, dd, 15.0/4.5
8_a	1.60, m	1.59, m	1.60, m	1.60, m	1.59, m
8_b	1.80, m	1.80, m	1.80, m	1.82, m	1.79, m
9	2.10, m	2.11, m	2.01, m	2.02, m	2.07, m
10	4.75, dd, 10.2/5.4	4.74, dd, 10.2/5.4	4.61, t, 6.6	4.66, t, 6.0	4.67, t, 6.0
11-OCH_3	3.64, s	3.64, s	3.64, s	3.65, s	3.64, s
10- OCH_3	3.34, s	3.30, s	3.310, s	3.30, s	-
10- OCH_3	-	-	3.316, s	-	-
Glucose moiety					
1`	4.69, d, 7.8	4.69, d, 7.8	4.69, d, 7.8	4.69, d, 7.8	4.69, d, 7.8
2`	3.20, dd, 9.0/7.8	3.21, dd, 9.0/7.8	3.23, dd, 9.0/7.8	3.21, dd, 9.0/7.8	3.22, dd, 9.0/7.8
3`	3.38, t, 9.0	3.38, t, 9.0	3.37, t, 9.0	3.37, t, 9.0	3.38, t, 9.0
4`	3.28, t, 9.0	3.27, t, 9.0	3.27, t, 9.0	3.28, t, 9.0	3.27, t, 9.0
5`	3.30, m	3.31, m	3.30, m	3.32, m	3.31, m
$6`_a$	3.67, dd, 12.0/6.0	3.68, dd, 12.0/6.0	3.67, dd, 12.0/6.0	3.67, dd, 12.0/6.0	3.68, dd, 12.0/6.0
$6`_b$	3.90, dd, 12.0/1.8	3.90, dd, 12.0/1.8	3.91, dd, 12.0/1.8	3.90, dd, 12.0/1.8	3.90, dd, 12.0/1.8
Phenylethyl Moiety					
$1``_a$	4.14, dd, 10.2/7.2	4.16, dd, 10.2/7.2	4.16, dd, 10.2/7.2	4.16, dd, 10.2/7.2	4.16, dd, 10.2/7.2
$1``_b$	4.20, dt, 10.2/7.2	4.20, dt, 10.2/7.2	4.21, dt, 10.2/7.2	4.22, dt, 10.2/7.2	4.22, dt, 10.2/7.2
2``	2.77, t, 7.2	2.83, t, 7.2	2.77, t, 7.2	2.77, t, 7.2	2.83, t, 7.2
4``	6.67, d, 1.8	7.06, d, 8.4	6.66, d, 1.8	6.66, d, 1.8	7.06, d, 8.4
5``	-	6.72, d, 8.4	-	-	6.72, d, 8.4
7``	6.69, d, 8.4	6.72, d, 8.4	6.69, d, 8.4	6.69, d, 8.4	6.72, d, 8.4
8``	6.55, dd, 8.4/1.8	7.06, d, 8.4	6.55, dd, 8.4/1.8	6.55, dd, 8.4/1.8	7.06, d, 8.4
Butyl moiety					
$1```_a$	-	-	-	3.45, m	3.54, t, 6.6
$1```_b$	-	-	-	3.60, m	-
2```	-	-	-	1.54, m	1.50, m
3```	-	-	-	1.39, m	1.38, m
4```	-		-	0.92, t, 7.2	0.92, t, 7.2

Table 2. ^{13}C NMR spectral data of compounds 1-5 (125 MHz, CD_3OD).

Position	1	2	3	4	5
Secoiridoid moiety					
1	98.48 d	98.48 d	98.36 d	98.37 d	98.30 d
3	154.20 d	154.20 d	154.10 d	154.10 d	154.10 d
4	110.60 s	110.60 s	110.60 s	110.00 s	110.50 s
5	30.76 d	30.73 d	30.90 d	30.78 d	30.77 d
6	36.62 t	36.58 t	36.65 t	36.67 t	36.54 t
7	174.40 s	174.40 s	174.40 s	174.40 s	174.40 s
8	34.88 t	34.87 t	30.96 t	31.26 t	35.02 t
9	37.29 d	37.38 d	37.05 d	37.17 d	37.38 d
10	104.20 d	104.20 d	104.50 d	103.70 d	103.40 d
11	168.90 s	168.90 s	168.80 s	168.90 s	168.90 s
11-OCH_3	51.79 q	51.78 q	51.82 q	51.82 q	51.78 q
10- OCH_3	54.0 q	54.0 q	53.52 q	53.32 q	-
10- OCH_3	-	-	54.06 q	-	-
Glucose moiety					
1`	100.70 d	100.70 d	100.70 d	100.70 d	100.60 d
2`	74.80 d	74.79 d	74.81 d	74.82 d	74.79 d
3`	78.39 d	78.39 d	78.44 d	78.44 d	78.39 d
4`	71.61 d	71.53 d	71.62 d	71.61 d	71.60 d
5`	77.96 d	77.90 d	77.97 d	77.98 d	77.95 d
6`	62.85 t	62.79 t	62.87 t	62.87 t	62.69 t
Phenylethyl Moiety					
1``	66.95 t	66.94 t	66.94 t	66.94 t	66.92 t
2``	35.37 t	35.13 t	35.38 t	35.39 t	35.13 t
3``	130.80 s	130.00 s	130.80 s	130.80 s	130.00 s
4``	116.50 d	130.90 d	116.50 d	116.40 d	130.90 d
5``	146.30 s	116.30 d	146.30 s	146.30 s	116.30 d
6``	144.90 s	157.10 s	145.0 s	143.0 s	157.10 s
7``	117.10 d	116.30 d	117.00 d	117.00 d	116.30 d
8``	121.30 d	130.90 d	121.30 d	121.20 d	130.90 d
Butyl Moiety					
1```	-	-	-	67.49 t	62.84 t
2```	-	-	-	33.09 t	35.81 t
3```	-	-	-	20.51 t	20.03 t
4```	-	-	-	14.29 q	14.24 q

showed UV absorptions at 235 and 282 nm, IR bands at 3375, 1710, 1625 and 1520 cm^{-1} and a ^1H NMR signals due to H-1 at δ 5.42, H-3 at δ 7.47, H-1` at δ 4.69, H-2`` at δ 2.77 and an ABX spin system at δ 6.66 (H-4``), 6.69 (H-7``) and 6.55 ( H-8``), all of which are features common to secoiridoid glucoside nucleous with 3,4-dihydroxyphenylethyl moiety. The ^1H NMR spectrum of **4** was very similar to that of **3** except it lacked a signal due to one of the methoxyl group at C-10 position and contained extra signals for an additional oxybutyl group indicated by the signals of one oxymethylene group protons at δ 3.45 and 3.60 (each, m), attributed to H-1_a``` and H-1_b```, respectively; two methylene protons at δ 1.54 and 1.39 (each 2H, each m) attributed to H-2``` and H-3```, respectively and one methyl triplet at δ 0.92 (*J* = 7.2 Hz, H-4```). The ^{13}C and DEPT NMR spectrum of **4** also showed a signals arising from an extra oxybutyl moiety at δ 67.49 t (C-1```), 33.09 t (C-2```), 20.51 t (C-3```) and 14.29 q (C-4```). Linkage of the oxybutyl moiety to C-10 position was comfirmed by the intense cross-peak in the HMBC spectrum between H-10 (δ 4.66) and the oxymethylene carbon at (δ 67.49) and the cross-peaks between H-1_a``` and H-1_b``` and C-10 at (δ 103.70), clearly indicated the location of the oxybutyl moiety at the 10-position of the secoiridoid moiety. Furthermore HMBC spectrum showed correlation between C-10 and the methoxyl protons at δ 3.30 and between H-10 and the methoxyl carbon at δ 53.32 indicating its acetal nature. Consequently compound **4** was determined to be ligustaloside A butyl-methyl acetal.

Compound **5**, was obtained as a white amorphous powder. The positive FAB mass spectrum of **5** showed an intense peak at *m/z* 637 corresponding to the $[M+Na]^+$ quasimolecular ion of **5**, indicating its $[M]^+$ to be 602 which corresponds to the molecular formula $C_{29}H_{42}O_{14}$. The UV absorptions and IR bands were very similar to those of **2**. Complete ^1H and ^{13}C NMR resonance assignments were carried out by 1D and 2D shift-correlated NMR techniques. COSY, HMQC and HMBC showed a signal pattern very similar to that of **2** except for the signal of the methoxyl group at C-10 position which was replaced by an oxybutyl group as in **4**. The presence of AA`BB` system of the *p*-hydroxyphenylethyl moiety is in close agreement with that of **2**; δ 6.72 (2H, d, *J* = 8.4 Hz, H-5``and H-7``) and δ 7.06 (2H, d, *J* = 8.4 Hz, H-4``and H-8``). The oxybutyl group was evidenced from the ^1H NMR signals at δ 3.54 (2H, t, *J* = 6.6 Hz, H_2-1```), 1.50 (2H, m, H_2-2```), 1.38 (2H, m, H_2-3```) and 0.93 (3H, t, *J* = 7.2 Hz, H_3-4```). The ^{13}C NMR spectrum of **5** contained 29 signals; comparison with the spectrum of **2** allowed eleven signals were assigned to secoiridoid aglycon moiety, eight signals to a *p*-hydroxyphenylethyl moiety, six signals to a *β*-glucopyranosyl moiety identical to those found in **2**, except the up field shift of C-10 of the secoiridoid moiety by about 1.0 ppm. The remaining four signals in the spectrum fitted well with an oxybutyl group with chemical shifts at δ 62.84, 35.81, 20.03 and 14.24 and were assigned to C-1```, 2```, 3``` and C-4```, respectively. The site of attachment of the oxybutyl group was established to be at C-10 position as deduced from HMBC correlations as in **4**. Based on the forementioned evidence, the chemical structure of **5** was elucidated to be ligustaloside B butyl hemiacetal.

Result of the hypotensive effect of the n-BuOH fraction and isolated new compounds 1-5:

A dose dependent hypotensive effect in the normal anaesthetized cats was obtained by administration of gradually increasing doses (0.5, 1.0, 2.0, 4.0, 8.0, 16.0 mg/ml) of the aqueuos *n*-BuOH fraction. adrenaline (5 µg/kg) was used for induction of hypertension of the anaesthetized cat which returned to normal level after 12 min. A mild drop of the hypertension to about 50% in 1 min. was observed after administration of the *n*-BuOH fraction (50 mg/kg) on the top of the hypertension. After administration of another dose of the *n*-BuOH fraction (50 mg/kg) the hypertension returned to normal level with about 2 min. Another dose of adrenalin (5 µg/kg) was used for induction of hypertension. The induced hypertension effect of adrenaline after administration of the *n*-BuOH fraction confirmed that this fraction not has α- or ß- blocking effect. Administration of the *n*-BuOH fraction with adrenaline reduces the hpertensive effect of adrenalin to about 40%, which indicat the antihypertensive effect of the *n*-BuOH fraction. The prominant hypotensive effect of the *n*-BuOH fraction after blocking successively the α- and ß- adrenergic and histaminergic receptors by injection of Regitine (2 mg/kg), propranolol (2 mg/kg) and pheniramine (1 mg/kg) and cimetidine (2 mg/kg) respectively before administration of the *n*-BuOH fraction in each case, and the antagonized hypotensive effect of the *n*-BuOH fraction after blocking muscarinic (cholinergic) receptors by injection of Atropine (1 mg/kg), clearly indicated that the hypotensive effect of the *n*-BuOH fraction was largely antagonized by anticholinergics and not affected by α- or ß-adrenergic or histaminergic blocking agents. Therefore, the mechanism for the

hypotensive effect of the *n*-BuOH fraction may be due to the cholinergic effect. Doses of (1.0, 2.0, 4.0, 8.0 mg/cat), of the new compounds **1-5** show a dose dependent hypotensive effect. Doses of (4 and 8 mg/cat) of compounds **1, 3** and **4** produced a mild hypotensive effect, while, doses of (4 and 8 mg/cat) of compounds **2** and **5** produced a very mild hypotensive effect. All compounds **1-5** with doses (1.0 and 2.0 mg/cat), produced no reduction in blood pressure.

Compound **1**. A white amorphous powder; UV λ_{max} (MeOH) nm: 235, 284; IR (KBr) cm^{-1}: 3375, 1715, 1625 and 1520. FAB mass spectrum: 588, *m/z* 611 $[M+Na]^+$ (calc. for $C_{26}H_{36}O_{15}$) ^1H and ^{13}C NMR data are shown in Tables 1 and 2.

Compound **2**. A white amorphous powder; UV λ_{max} (MeOH) nm: 231, 281; IR (KBr) cm^{-1}: 3400, 1720, 1630 and 1520. FAB mass spectrum: 572, *m/z* 595 $[M+Na]^+$ (calc. for $C_{26}H_{36}O_{14}$) ^1H and ^{13}C NMR data are shown in Tables 1 and 2.

Compound **3.** A white amorphous powder; UV λ_{max} (MeOH) nm: 234, 282; IR (KBr) cm^{-1}: 3380, 1710, 1630 and 1520. FAB mass spectrum: 602, *m/z* 625 $[M+Na]^+$ (calc. for $C_{27}H_{38}O_{15}$) ^1H and ^{13}C NMR data are shown in Tables 1 and 2.

Compound **4**. A white amorphous powder; UV λ_{max} (MeOH) nm: 235, 282; IR (KBr) cm^{-1}: 3375, 1710, 1625 and 1520. FAB mass spectrum: 644, *m/z* 667 $[M+Na]^+$ (calc. for $C_{30}H_{44}O_{15}$) ^1H and ^{13}C NMR data are shown in Tables 1 and 2.

Compound **5.** A white amorphous powder; UV λ_{max} (MeOH) nm: 231, 281; IR (KBr) cm^{-1}: 3400, 1720, 1625 and 1520. FAB mass spectrum: 614, *m/z* 637 $[M+Na]^+$ (calc. for $C_{29}H_{42}O_{14}$) ^1H and ^{13}C NMR data are shown in Tables 1 and 2.

ACKNOWLEDGMENTS

We express our appreciation to Prof. Dr. Jhon, P.N. Rosazza, Head, Division of Medicinal and Natural Products Chemistry and Director of the Center for Biocatalysis and Bioprocessing, College of Pharmacy, University of Iowa, Iowa City, IA 52242 USA, for your measurement of the NMR and MS spectra.

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